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[Enka Jifueniru no Dobutsu Soshiki Kara no Chushutsu Teiryoho Narabi ni Shiroezumi ni Okeru Tainai Bunpu]

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# THE ISOLATION AND DETERMINATION OF DIPHENYL CHLORIDE FROM ANIMAL TISSUE AND ITS DISTRIBUTION IN WHITE RATS

## Foreword

On the basis of subsequent surveys and studies, it is believed that the cause of the rice bran oil disease (called oil disease below) that broke out across Western Japan, but was centered in the Kitakyushu region, during the summer and fall of 1968, was probably the intermixture of diphenyl chloride (trade name Kaneclor), which was used as a heating medium in the deodorization process in the manufacture of rice bran oil at the Kanemi Warehousing Company, Limited, (abbreviated as Kanemi rice bran oil, below) in Kitakyushu City [1, 11].

Because the chemical structure of diphenyl chloride is a mixture of various compounds which have a diphenyl backbone, with chlorine bonded in different positions and in differing numbers, it can normally be divided into approximately 15 components with a gas chromatograph fitted with an electron capturing detector (abbreviated as ECD, below). Because the relative sensitivity for each component differs, it is not always possible to classify all of them. For this reason, it is impossible to make an accurate determination of diphenyl chloride with ECD at the present time.

The writers made a study of methods for extracting diphenyl chloride from animal tissue and excreta and studied methods of determining diphenyl

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chloride by measuring the intensity of the characteristic x-rays of chlorine using x-ray fluorescense analysis and obtained excellent results.

Diphenyl chloride is a fat-soluble, chemically stable compound that is classified as an aromatic hydrocarbon containing chlorine. For this reason, it is postulated that it accumulates to a high degree within the body. I.e. the danger of the chronic toxicity of the diphenyl chloride that is taken up by the body must also be considered [12].

At the present time, such organochloride agricultural pharmaceuticals as DDT, BHC, and Dorin [Tr. Note: Phonetic in the original], which have a chemical structure quite similar to that of diphenyl chloride, have become an important problem in food sanitation. For this reason, the writers thought that a study of the distribution of diphenyl chloride within the living body would have major significance, not only from the viewpoint of studying oil disease, but also that it would provide knowledge in the studies of residual agricultural chemicals. Accordingly, they made a study of the distribution of diphenyl chloride in white rats obtaining two or three bits of knowledge which are reported here.

## Experimental Methods

### 1. X-Ray Fluorescence Analysis.

The x-ray fluorescence analysis for the determination of diphenyl chloride was performed under the following measurement conditions.

Equipment: A Rigaku Denki manufactured, x-ray fluorescence analyzer, Geigerflex D-9C Model KD-4.

X-ray tube anticathode: Cr

Imposed voltage: 40 VVP

Current: 30 mA

Spectral monocrystal: Ge

Detector: Gas flow type, proportional counter

X-ray path: He gas stream

Bragg angle: (2 θ) 92.7° (primary line of the Cl kα line)

2. Gas Chromatography.

The qualitative analysis of the diphenyl chloride by gas chromatography was accomplished under the following conditions.

Equipment: Shimazu Company gas chromatograph Model GC-3A E

Detector: ECD (Irradiation source $^{3}H$)

Column: Internal diameter 4 mm, length 1.2 m (glass)

Packing: 5% DC-11/Chromosorb G

(AW-DMCS) (60 - 80 mesh)

Column temperature, detector temperature, injection port temperature: 215°, in all cases.

3. Experimental Animals.

Male Wistar white rats weighing 280 - 320 g were used.

4. Samples and Methods of Administration.

a. Control rice bran oil: Normal Kanemi rice bran oil containing absolutely diphenyl chloride.

b. Rice Bran Oil with diphenyl chloride added: The same Kanemi rice bran oil as the control, to which 2,500 ppm of diphenyl chloride (Kanechlor

-400) had been added.

c. Poisoned rice bran oil: Kanemi rice bran oil with the date of manufacture of February 5, 1968 (diphenyl chloride content 2,500 ppm).

The previously described experimental animals were divided into three groups and each group was forceably administered one 2 ml dose of the above samples using a gastric sonde.

5. Isolation of Diphenyl Chloride from Animal Tissue and Excreta.

After administering the samples, the tissues, organs and excreta of the white rats were finely chopped (blood serum and urine untreated) and homogenized in 10 times the quantity of a 50 ethanol solution of 1 N potassium hydroxide. The homogenate was refluxed by heating for two hours over a boiling water bath. After cooling, the samples were extracted three times with 100 ml of n-hexane, each time, then washed. The residue, after drying with anhydrous sodium sulfate and removing the solvent by low pressure distillation, was dissolved in 2 ml of n-hexane and each sample divided into two equal parts. One part was used for the quantitative determination of diphenyl chloride by x-ray fluorescence analysis, while the other was suitably diluted and used for the qualitative analysis by ECD.

## Results

1. Calibration Curves for Diphenyl Chloride by X-Ray Fluorescence Analysis.

The calibration curve shown in Figure 1 was obtained for diphenyl chloride contents between the limits of 20 - 1,000 μg when the logarithms of the diphenyl chloride content were placed on the horizontal axis and the

counting rates for the intensity of the characteristic X-ray intensities for chlorine were placed on the vertical axis and the two plotted against each other.

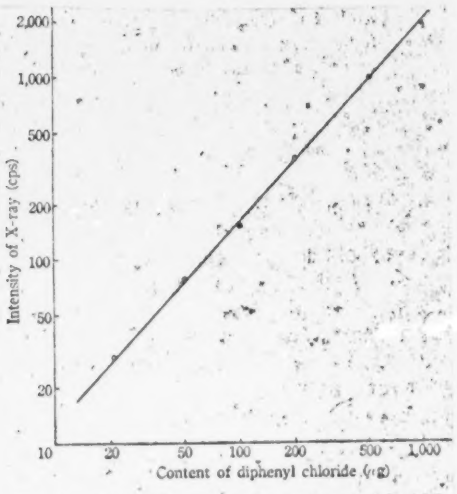


Fig. 1. Calibration curve of diphenyl chloride by X-ray fluorescence analysis

Subsequently, a calibration curve was prepared before each measurement and the diphenyl chloride content determined from the calibration curve involved.

2. Diphenyl Chloride Addition and Recovery Experiments.

One ml of a n-hexane solution containing 200 μg of diphenyl chloride was added to the excrement and organ tissue of white rats that had been fed a normal solid feed. Subsequently, diphenyl chloride was extracted from these specimens and a quantitative determination made. Table 1 shows the results for a determination of the recovery rate.

Table 1. Recovery of Diphenyl Chloride Added to Tissues and Organs of Rats

| Tissue or organ | Recovery | |
|---|---|---|
| | (μg) | (%) |
| Skin | 150 | 75 |
| Muscles | 140 | 70 |
| Liver | 130 | 75 |
| Kidneys | 140 | 70 |
| Spleen | 190 | 95 |
| Pancreas | 190 | 95 |
| Heart | 180 | 90 |
| Lungs | 140 | 70 |
| Brain | 130 | 65 |
| Genitals | 140 | 70 |
| Blood | 180 | 90 |
| Stomach | 150 | 75 |
| Intestines | 150 | 75 |
| Urine | 190 | 95 |
| Feces | 180 | 90 |
| Diphenyl chloride only | 190 | 95 |

200 μg of diphenyl chloride were added into each tissue or organ. Each value represents the average of the five animals

There were good recovery rates for diphenyl chloride alone and from the pancreas, heart, spleen, blood, urine and feces, but the recovery from the skin, muscle, liver, lungs, brain, genitals, stomach and intestines was below 80%. Accordingly, the diphenyl chloride content was determined after correction for the respective recovery rates in the subsequent experiments.

3. Diphenyl Chloride Excretion Tests.

White rats were divided into three groups of three rats in each group. Two ml of the previously discussed control rice bran oil, rice bran oil with diphenyl chloride added and poisoned rice bran oil was administered orally to each specimen in each of the respective groups and a determination made of the urine and feces 24 hours and 48 hours later.

The quantity of diphenyl chloride administered to each specimen in

the diphenyl chloride-added rice bran oil group and the poisoned rice bran oil group was 5 mg.

Table 2. Content of Diphenyl Chloride in Excreta of Rats

| Excreta | Administered rice oil | Amount of excreted DC ($\mu$g) | | | % of excretion to dose |
|---|---|---|---|---|---|
| | | 24 hr | 48 hr | Total | |
| Feces | Rice oil added DC | 530 | 176 | 706 | 14 |
| | Poisonous rice oil | 460 | 108 | 568 | 11 |
| Urine | Rice oil added DC | 0 | 0 | 0 | 0 |
| | Poisonous rice oil | 0 | 0 | 0 | 0 |

DC : Diphenyl chloride (Kanechlor 400)
5 mg of diphenyl chloride were administered to each animal.
Each value represents the average of three animals.

As is clear from Table 2, 11% and 9% of the diphenyl chloride administered was excreted in the feces 24 hours after the administration of diphenyl chloride in the diphenyl chloride-added rice bran group and the poisoned rice bran oil group, respectively. The respective excretion rates 24 hours afterwards were 14% and 11%.

Because the peaks of the patterns in the ECD gas chromatograms of the extract from this excreta were in virtual agreement with those at the time of administration, it is believed that the diphenyl chloride in the feces was excreted without absorption and without modification.

Absolutely no diphenyl chloride was detected by x-ray fluorescence analysis or ECD in the urine of both groups.

4. Distribution of Diphenyl Chloride in the Body.

Control rice bran oil, diphenyl chloride-added rice bran oil and

poisoned rice bran oil were administered orally once daily for seven days continuously in amounts of 2 ml to each of the three specimens in each of the respective groups. The diphenyl chloride content in organ tissue was then measured giving the results shown in Table 3.

Table 3. Distribution of Diphenyl Chloride in Tissues and Organs of Rats

| Tissue or organ | Rice oil DC added | | Poisonous rice oil | |
|---|---|---|---|---|
| | (μg) | (ppm) | (μg) | (ppm) |
| Skin | 2,250 | 430 | 2,500 | 490 |
| Muscles | 1,300 | 105 | 1,170 | 105 |
| Liver | 420 | 30 | 360 | 25 |
| Kidneys | 50 | 20 | 30 | 10 |
| Spleen | 0 | 0 | 0 | 0 |
| Pancreas | 245 | 165 | 225 | 155 |
| Heart | 0 | 0 | 0 | 0 |
| Lungs | 145 | 100 | 150 | 95 |
| Brain | 0 | 0 | 0 | 0 |
| Genitals | 0 | 0 | 0 | 0 |
| Blood | 0 | 0 | 0 | 0 |
| Stomach | 55 | 15 | 60 | 20 |
| Intestines | 650 | 145 | 700 | 160 |

DC : Diphenyl chloride (Kanechlor 400)
35 mg of diphenyl chloride was administered to each animal as total dose.
Each value represents the average of the three animals.

No organochloride was observed in any of the organ tissues from the white rats in the control group.

The internal distribution of diphenyl chloride in both the diphenyl chloride-added rice bran oil group and the poisoned rice bran oil group showed the greatest accumulation in the skin, followed by the muscle, the intestine, the liver, the pancreas and the lungs. The quantity accumulated per g of wet weight by organ was in the order skin, spleen, intestine, muscle and lung.

The reason why there is a high diphenyl chloride content in the intestine is because the intestine contains unabsorbed and unexcreted unmodified,

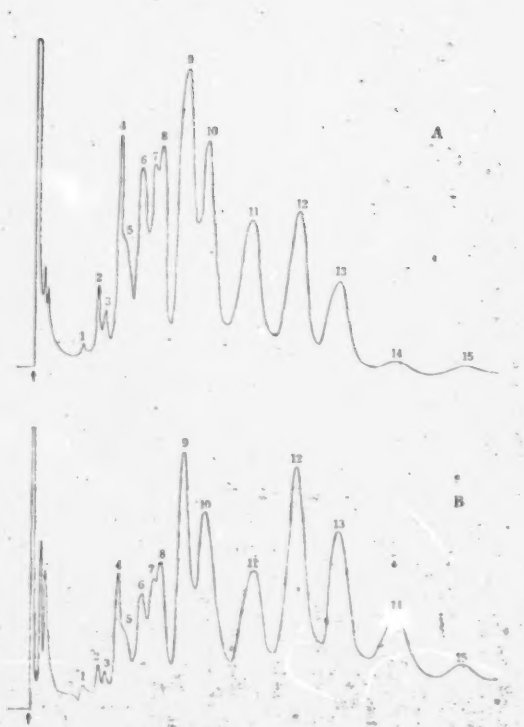


Fig. 2. Gas-chromatogram of diphenyl chloride and extract from poisonous rice oil
A : Diphenyl chloride (Kanechlor 400)
B : Extract from poisonous rice oil

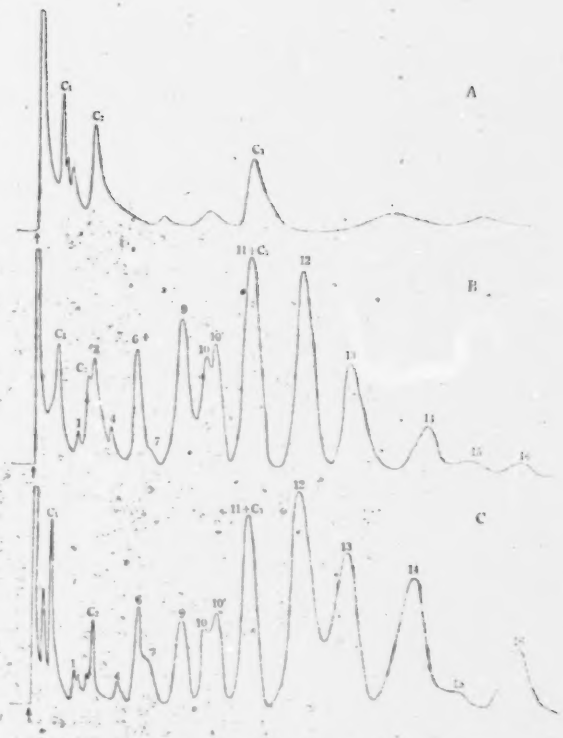


Fig. 3. Gas-chromatogram of extract from liver of rat administered with diphenyl chloride
A : Control
B : Administered with diphenyl chloride added rice oil
C : Administered with poisonous rice oil

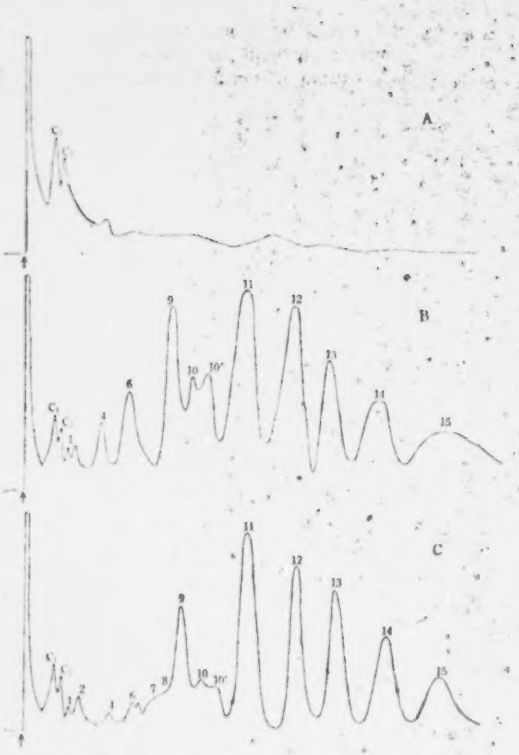


**Fig. 4.** Gas-chromatogram of extract from muscles of rat administered with diphenyl chloride
A, B and C : the same as shown in Fig. 3

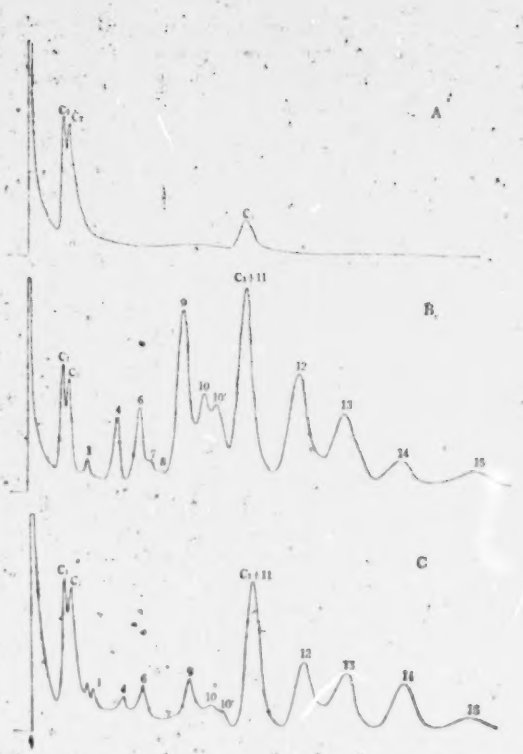


**Fig. 5.** Gas-chromatogram of extract from skin of rat administered diphenyl chloride
A, B and C : the same as shown in Fig. 3

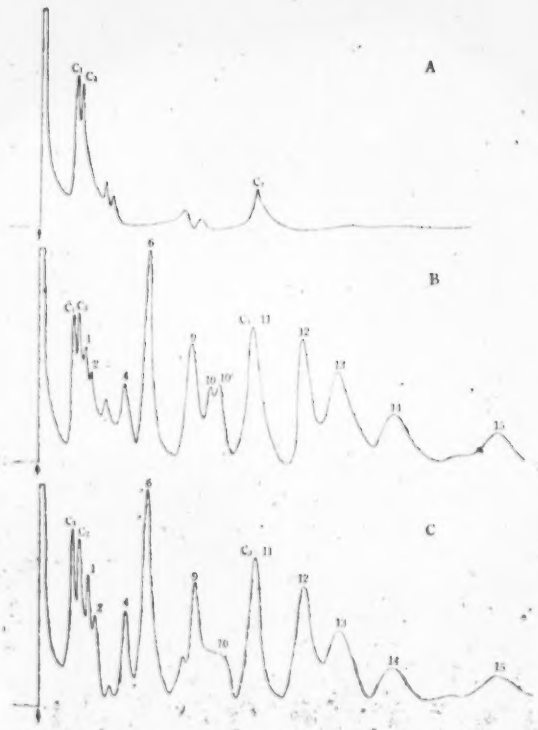


**Fig. 6.** Gas-chromatogram of extract from kidneys of rat administered diphenyl chloride
A, B and C: the same as shown in Fig. 3

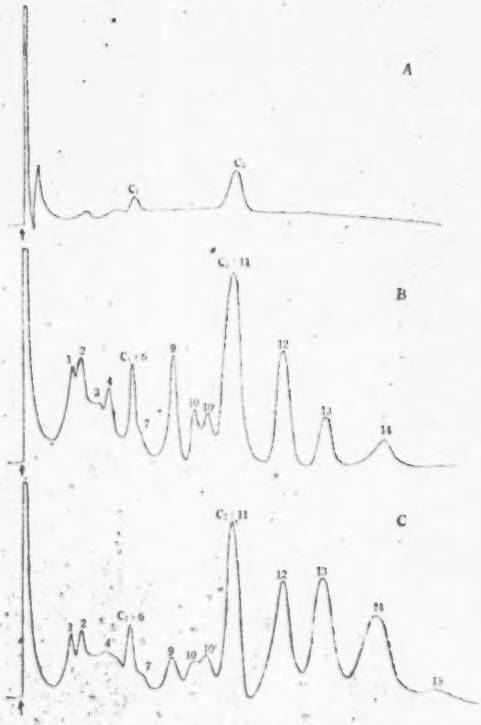


**Fig. 7.** Gas-chromatogram of extract from lungs of rat administered with diphenyl chloride
A, B and C: the same as shown in Fig. 3

residual diphenyl chloride.

The fact that the accumulation of diphenyl chloride in the skin and in the muscle, particularly in the skin, is consistent with the fact that marked skin symptoms were observed in the Kanemi rice bran oil poisoning cases [1,3,4,8,12].

Because the deterioration limit for diphenyl chloride by x-ray fluorescence analysis is 20 μg, it was not possible to make a quantitative determination for the spleen, genital organs and blood, but the presence of diphenyl chloride in these organs, although in microscopic quantities, was observed in ECD. No diphenyl chloride was observed in the brain either by x-ray fluorescence analysis or by ECD.

In addition, no clear difference was observed between the diphenyl chloride-added rice bran oil group and the poisoned rice bran oil group in any of the tissues.

5. The Composition of the Diphenyl Chloride in Each Type of Tissue

A comparative study was made of the ECD patterns for the extracts from the tissues in each of the previously described three groups.

On comparing the ECD patterns for the n-hexane extract for the poisoned rice oil and those for diphenyl chloride (Kanechlor-400), as shown in Figure 2 those with as short retention times as 4, 6, 7 and 8 are small while, in contrast, those with a long retention time, 11, 12, 13 and 14 are greater. This is believed to be because the low boiling point components of diphenyl chloride were driven off by the operations subsequent to the deodorizing treatment during the rice bran oil manufacturing process.

(a) Liver.

The ECD patterns for the extracts from the white rat livers of each of the three groups are as shown in Figure 3.

In both the diphenyl chloride-added rice bran oil group and the poisoned rice bran oil groups, unlike the respective substances administered (reference Fig. 2) peaks 3, 5, and 8 had disappeared and a new peak, 10', was observed between 10 and 11. In addition $C_1$, $C_2$ and $C_3$ are substances with electron affinity from the liver itself that are unrelated to diphenyl chloride.

(b) Muscles and Skin.

The ECD patterns for the extracts from muscle and skin are shown in Figure 4 and Figure 5, respectively. Like the liver, peaks 3, 5 and 8 had disappeared in both cases and a new peak 10' was observed.

Further, because all of the peaks with retention times lower than 8 were much smaller than was the case in the liver, it is believed that the low-boiling point substances do not migrate to the skin and muscles.

(c) Kidneys.

As shown in Figure 6, peak 6 was observed to be much higher in the kidneys than in the other tissue.

(d) Lungs.

In the case of the lungs, peaks 5 and 8 disappeared, as shown in Figure 7, and the new peak 10' was observed.

(e) Brain.

As shown in Figure 8, the results for the brain differed markedly from those for the other types of tissue. In the control group, as well, a large number of substances having an electron affinity were observed, as were a large number of peaks having particularly short retention times. A number of these had retention times that were in agreement with those of the objective diphenyl chloride. However, because the relative retention times and the relative heights of the peaks among the control group, the diphenyl chloride added rice bran oil group and the poisoned rice bran oil group were generally in agreement, it is believed that the peaks in the ECD for the extracts from the brain did not derive from vinyl chloride, but represent substances that are intrinsic to the brain that have electron affinity.

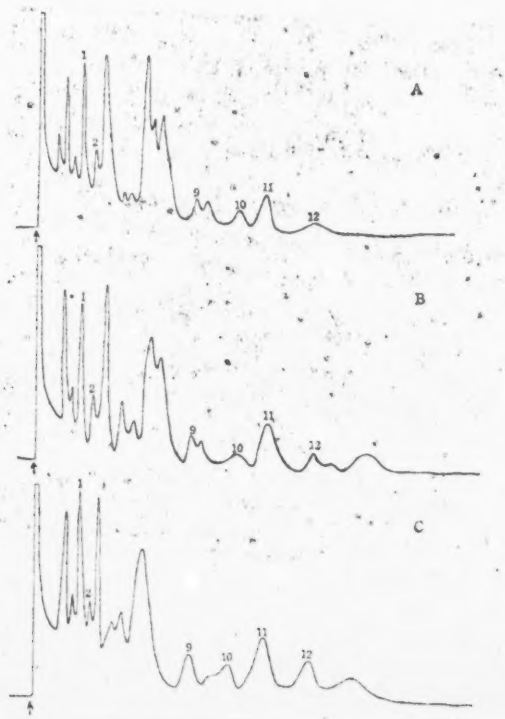


Fig. 8. Gas-chromatogram of extract from brain of rat administered diphenyl chloride
A, B and C: the same as shown in Fig. 3

From the fact that the compositional ratios of diphenyl chloride from each of the types of tissue differed, it is believed that the absorption, the migration properties within the body and the modality of metabolism and excretion of each of the components in the diphenyl chloride that was administered differ in white rats.

No clear difference was observed in any of the ECD patterns for all of the tissues between the diphenyl chloride-added rice bran oil group and the poisoned rice bran oil group.

## Conclusions

(1) Up to 48 hours after a single oral dose of 2 ml of rice bran oil containing 2,500 ppm of diphenyl chloride to white rats, approximately 10% of the diphenyl chloride administered was excreted in the feces, however absolutely none was observed in the urine.

(2) When rice bran oil containing 2,500 ppm of diphenyl chloride was administered to white rats once daily at a dosage of 2 ml each time for seven days continuously, it was noted that distribution of diphenyl chloride in the body showed the greatest accumulation in the skin, followed by the muscle, intestine, liver, pancreas and lungs. It was not possible to detect any in the heart, spleen, genital organs and blood by x-ray fluorescence analysis, however minute quantities were observed by ECD. Absolutely none was observed in the brain by either method.

(3) From the fact that there were differences in the diphenyl chloride compositional ratios in the ECD gas chromatograms of extracts from the tissue of white rats that had been administered diphenyl chloride, it is believed that the modality of accumulation within the body, metabolism and excretion

of each of the components of the diphenyl chloride administered differs.

In conclusion, deep thanks are expressed to Dr. Yoshio Ikeda, the Chief of the Toxicity Division of this laboratory for his valuable advice during the present study. In addition, thanks are expressed to Miss Tsuruko Shioaki and Miss Kumiko Kitsuki for their cooperation in the gas chromatography analyses.

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BIBLIOGRAPHY


1. Sugiyama, Shokan, Shokuhin Eisei Kenkyu [Food Sanitation Research], Vol. 19, 1969, p. 783.

2. Tsukamoto, Hisao, et al., The Kyushu University Oil Disease Research Section, Analysis Subsection, Fukuoka Igaku Zasshi [Fukuoka Medical Journal], Vol. 60, 1969, p. 496.

3. Tsukamoto, Hisao, Yoshimura, Tsuneyoshi, Eisei Kagaku [Sanitary Chemistry], Vol. 15, 1969, p. 213.

4. Inagami, Kaoru, Koga, Tomihide, Tomita, Yasuichiro, Shokueishi [Food Sanitation Journal], Vol. 10, 1969, p. 312.

5. Akitani, Toshimi, Ibid., Vol. 10, 1969, p. 307.

6. Sekida, Hiro, Uchiyama, Sadao, et. al., Eisie Shiho [Sanitation Experimental Report], Vol. 87, 1969, p. 20.

7. Tabuchi, Hiro, et al., "Compileation of Research Reports Based on Fiscal Year 1968 Science and Technology Agency Special Research Promotion Coordination Funds," To Be Published.

8. Matano, Kagemaru, Rinsho Eiyo [Clinical Nutrition], Vol. 35, 1969, p. 34.

9. Kawakita, Heizo, Mizutani, Yasuhisa, Seikatsu Eisei [Life Sanitation], Vol. 13, 1969, p. 2.

10. Fukushima, Narihiko, Kashimoto, Ryu, Kobayashi, Taro, Annals of the 17th Technical Lecture Meeting of the Japan Food Sanitation Society, 1969, p. 16.

11. Ogata, Tadamori, Kinan, Tomiyoshi, et al., Nihon Koeishi [Japanese Journal of Public Health], Vol. 16, 1969, p. 647.

12. Nomura, Shigeru, Yumoto, Narishige, Seikatsu Eisei, Vol. 13, 1969, p. 11.

13. Oshiba, Keiichi, Okada, Toshio, et al., Annals of the 17th Technical Lecture Meeting of the Japan Food Sanitation Society, 1969, p. 17.